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(54) Title: USE OF A PEPTIDE

(57) Abstract

GLP-1(7-37), GLP-1(7-36)amide, and certain related compounds can be used to provide a medicament for use in the treatment of diabetes in a regimen which additionally comprises treatment with an oral hypoglycaemic agent. A strong synergistic effect is observed.

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USE OF A PEPTIDE

FIELD OF THE INVENTION

The present invention relates to the use of GLP-1(7-37), GLP-1(7-36)amide, or certain related compounds for the preparation of a medicament for use in the treatment of diabetes in a regimen which additionally comprises treatment with an oral hypoglycaemic agent. The invention also relates to a method of treating diabetes by using said medicament.

BACKGROUND OF THE INVENTION

Diabetes is characterized by an impaired glucose metabolism manifesting itself among other things by an elevated blood glucose level in the diabetic patients. Underlying defects lead to a classification of diabetes into two major groups: type 1 diabetes, or insulin demanding diabetes mellitus (IDDM), which arises when patients lack ß-cells producing insulin in their pancreatic glands, and type 2 diabetes, or non-insulin dependent diabetes mellitus (NIDDM), which occurs in patients with an impaired ß-cell function besides a range of other abnormalities.

Type 1 diabetic patients are currently treated with insulin, while the majority of type 2 diabetic patients are treated either with agents that stimulate ß-cell function or with agents that enhance the tissue sensitivity of the patients towards insulin.

Among the agents applied for stimulation of the ß-cell function, those acting on the ATP-dependent potassium channel of ß-cells are most widely used in current therapy. The so-called sulfonylureas such as tolbutamide, glibenclamide, glipizide, and gliclazide are used extensively and other agents such as AG-EE 623 ZW also acting at this

molecular site are under development (AG-EE 623 ZW is a company code for (S)-(+)-2-ethoxy-4-[2-[[3-methyl-1-[2-(1-piperidinyl)phenyl]butyl]-amino]-2-oxoethyl]benzoic acid, a compound described in European patent publication No. 147,850 (to Dr. Karl Thomae GmbH)). Among the agents applied to enhance tissue sensitivity towards insulin metformin is a representative example.

Even though sulfonylureas are widely used in the treatment of NIDDM this therapy is, in most instances, not satisfactory: In a large number of NIDDM patients sulfonylureas do not suffice to normalize blood sugar levels and the patients are, therefore, at high risk for acquiring diabetic complications. Also, many patients gradually lose the ability to respond to treatment with sulfonylureas and are thus gradually forced into insulin treatment. This shift of patients from oral hypoglycaemic agents to insulin therapy is usually ascribed to exhaustion of the ß-cells in NIDDM patients.

Over the years, numerous attempts have therefore been made to provide novel agents which stimulate ß-cell function in order to offer the NIDDM patients an improved treatment. Recently, a series of peptides derived from glucagon-like peptide-1 have been considered as insulinotropic agents for therapeutic use.

Glucagon-like peptide-1, also referred to as GLP-1, is a peptide sequence found in the C-terminal portion of mammalian proglucagon. Prior to 1985, no definite biological activity of GLP-1 had been reported. However, in 1985 it was demonstrated that the amide of a fragment of GLP-1, namely GLP-1(1-36)amide, stimulates insulin release from isolated precultured rat pancreatic islets in the presence of glucose in a dose-dependent manner (Schmidt, W.E. et al. Diabetologia **28** (1985) 704-7). This finding suggests that GLP-1(1-36)amide and related peptides might be useful in the treatment of type 2 diabetes. Due to its substantially closer sequence homology to glucagon and glucosedependent insulinotropic peptide, also referred to as GIP, Schmidt et

al. suggested that an even stronger glucagon- and/or GIP-like biological activity could be expected with GLP-1(7-36) than with the intact peptide. In recent years, particular interest has focused on the GLP-1 fragments GLP-1(7-37) and GLP-1(7-36)amide and analogues and functional derivatives thereof. The designation GLP-1(1-36) indicates that the peptide fragment in question comprises the amino acid residues from (and including) number 1 to (and including) number 36 when counted from the N-terminal end of the parent peptide, GLP-1. Similarly, the designation GLP-1(7-37) designates that the fragment in question comprises the amino acid residues from (and including) number 7 to (and including) number 37 when counted from the N-terminal end of the parent peptide, GLP-1. The amino acid sequence of GLP-1(7-36)amide and of GLP-1(7-37) is given in formula I:

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-
Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-
Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-X

(I)

which shows GLP-1(7-36)amide when X is NH_2 and GLP-1(7-37) when X is Gly-OH.

That GLP-1(7-36)amide is indeed an insulinotropic agent in man has been demonstrated by Kreymann, B. et al. who infused this peptide into healthy volunteers and observed a significant rise in plasma insulin (Lancet **2** (1987) 1300-304).

The insulinotropic action of GLP-1(7-37) in diabetic as well as in nondiabetic subjects has been demonstrated by Nathan, D.M. et al. Diabetes Care **15** (1992) 270-76.

International Patent Application No. WO 87/06941 (to The General Hospital Corporation) relates to a peptide fragment which comprises GLP-1(7-37) and functional derivatives thereof and to its use as an insulinotropic agent.

International Patent Application No. 90/11296

(to The General Hospital Corporation) relates to a peptide fragment which comprises GLP-1(7-36) and functional derivatives thereof and has an insulinotropic activity which exceeds the insulinotropic activity of GLP-1(1-36) or GLP-1 (1-37) and to its use as an insulinotropic agent.

International Patent Application No. 91/11457 (to Buckley et al.) relates to effective analogs of the active GLP-1 peptides 7-34, 7-35, 7-36, and 7-37.

The effect of GLP-1(7-37) in combination with glibenclamide on insulin secretion from rat pancreatic islets was studied in vitro by Parker, J.C. et al. (Diabetes **40** (suppl. 1) (1991) 237 A). Only an **additive** effect of the two agents was observed.

However, to the best of the knowledge of the present inventors the surprising synergistic effect in vivo achieved by the combined use of an oral hypoglycaemic agent and a fragment of GLP-1 or an analogue or a functional derivative thereof has not previously been disclosed.

SUMMARY OF THE INVENTION

The present invention relates to the surprising finding that when GLP-1 related peptides are administered in combination with oral hypoglycaemic agents in general and with sulfonylureas in particular for treatment of type 2 diabetes, a synergistic effect is observed. This surprising observation has been made even in type 2 diabetic patients who fail to respond when sulfonylureas are administered alone.

Thus, in its broadest aspect the present invention relates to the use of GLP-1(7-37), GLP-1(7-36)amide, or a pharmaceutically acceptable peptide containing a fragment of the GLP-1(7-37) sequence, or an analogue or a functional derivative of such a peptide for the preparation of a medicament for use in the treatment of type 2 diabetes in a regimen which additionally comprises treatment with an oral

hypoglycaemic agent and to a method of treating type 2 diabetes which method comprises administering an effective amount of GLP-1(7-37), GLP-1(7-36)amide, or a pharmaceutically acceptable peptide containing a fragment of the GLP-1(7-37) sequence, or an analogue or a functional derivative of such a peptide to a patient in a regimen which additionally comprises treatment with an oral hypoglycaemic agent.

In a first preferred embodiment, the present invention relates to the use of GLP-1(7-36)amide for the preparation of a medicament for use in the treatment of type 2 diabetes in a regimen which additionally comprises treatment with an oral hypoglycaemic agent.

In a further preferred embodiment, the present invention relates to the use of GLP-1(7-37) for the preparation of a medicament for use in the treatment of type 2 diabetes in a regimen which additionally comprises treatment with an oral hypoglycaemic agent.

In a further preferred embodiment, the present invention relates to the use of an analogue of GLP-1(7-37) for the preparation of a medicament for use in the treatment of type 2 diabetes in a regimen which additionally comprises treatment with an oral hypoglycaemic agent.

In a further preferred embodiment, the present invention relates to the use of a functional derivative of GLP-1(7-37) for the preparation of a medicament for use in the treatment of type 2 diabetes in a regimen which additionally comprises treatment with an oral hypoglycaemic agent.

In a further preferred embodiment, the present invention relates to the use of GLP-1(7-37) or a fragment thereof or an analogue or a functional derivative of any of these including GLP-1(7-36)amide for the preparation of a medicament for use in the treatment of type 2 diabetes in a regimen which additionally comprises treatment with tolbutamide.

In a further preferred embodiment, the present

invention relates to the use of GLP-1(7-37) or a fragment thereof or an analogue or a functional derivative of any of these including GLP-1(7-36)amide for the preparation of a medicament for use in the treatment of type 2 diabetes in a regimen which additionally comprises treatment with glibenclamide.

In a further preferred embodiment, the present invention relates to the use of GLP-1(7-37) or a fragment thereof or an analogue or a functional derivative of any of these including GLP-1(7-36)amide for the preparation of a medicament for use in the treatment of type 2 diabetes in a regimen which additionally comprises treatment with glipizide.

In a further preferred embodiment, the present invention relates to the use of GLP-1(7-37) or a fragment thereof or an analogue or a functional derivative of any of these including GLP-1(7-36)amide for the preparation of a medicament for use in the treatment of type 2 diabetes in a regimen which additionally comprises treatment with gliclazide.

In a further preferred embodiment, the present invention relates to the use of GLP-1(7-37) or a fragment thereof or an analogue or a functional derivative of any of these including GLP-1(7-36)amide for the preparation of a medicament for use in the treatment of type 2 diabetes in a regimen which additionally comprises treatment with a biguanide.

In a further preferred embodiment, the present invention relates to the use of GLP-1(7-37) or a fragment thereof or an analogue or a functional derivative of any of these including GLP-1(7-36)amide for the preparation of a medicament for use in the treatment of type 2 diabetes in a regimen which additionally comprises treatment with metformin.

In a further preferred embodiment, the present invention relates to the use of GLP-1(7-37) or a fragment

thereof or an analogue or a functional derivative of any of these including GLP-1(7-36)amide for the preparation of a medicament for use in the treatment of type 2 diabetes in a regimen which additionally comprises treatment with (S)-(+)-2-ethoxy-4-[2-[[3-methyl-1-[2-(1-piperidinyl)phenyl]butyl]-amino]-2-oxoethyl]benzoic acid.

In this specification, analogues of GLP-1(7-37) or of GLP-1(7-36)amide, respectively, means peptides which differ from GLP-1(7-37) or from GLP-1(7-36)amide, respectively, in that at least one of the amino acid residues of GLP-1(7-37) or of GLP-1(7-36)amide, respectively, independently has been exchanged by another amino acid residue, preferably one which can be coded for by the genetic code. The definition also comprises the case when amino acid residues are added at or deleted from the N-terminal and/or the C-terminal end of the peptide. Preferably, the total number of such additions, deletions and exchanges does not exceed five, more preferred it does not exceed three.

DETAILED DESCRIPTION OF THE INVENTION

As mentioned above, patients treated with sulfonylureas gradually fail to respond to sulfonylurea treatment. It is generally accepted among those skilled in the art that this failure is due to exhaustion of ß-cells which, accordingly, are unable to excrete insulin in response to glucose stimulation. Also, it is generally accepted that the efficacy of sulfonylureas is limited by the capacity of ß-cells to produce and excrete insulin. Accordingly, one would not expect any additional therapeutic advantage by treating NIDDM patients with sulfonylureas and other agents stimulating ß-cell function as well.

Our finding that NIDDM patients may advantageously be treated with GLP-1 related peptides in combination

with sulfonylureas or other oral hypoglycaemic agents is therefore, indeed, surprising. In fact, we have found that concomitant treatment with oral hypoglycaemic agents and GLP-1 related peptides results in a synergistic response by the NIDDM patients: treatment with oral hypoglycaemic agents and GLP-1 related peptides gives rise to a metabolic response greater than the sum of the responses of either agents when applied alone. Even in cases of sulfonylurea failures, the oral agents have been found to significantly enhance efficacy of GLP-1 related peptides.

Combined treatment with GLP-1 related peptides and oral hypoglycaemic agents is thus novel, therapeutically useful, and surprising. Unforeseen, therapeutic advantages can be gained by treating the NIDDM patients with both types of drugs.

Among the GLP-1 related peptides that can thus be used in the treatment of type 2 diabetes GLP-1(7-37) and GLP-1(7-36)amide are particularly advantageous, as they are identical to the naturally occurring hormones. Shorter peptides comprising part of the GLP-1(7-37) sequence or analogues of such shorter peptides or analogues of GLP-1(7-37) itself or functional derivatives of any of these can also be used to advantage, since pharmacodynamic and pharmacokinetic properties can be changed according to patients' demand by modifying the GLP-1 related fragment.

The GLP-1 related peptides can be administered by methods currently available according to the invention for administration of peptides. Nasal application is particularly advantageous from a patient complience point of view. Details in this respect can be found in our copending Danish patent application No. DK 0364/92 relating to nasal administration of medicaments comprising GLP-1 related peptides which was filed simultaneously with the present application. The contents of said application is hereby incorporated in its entirety by reference. Administration by injection or infusion will be preferred in instances where a specific protracted plasma profile of the active peptide is

required, and oral administration is preferred in instances where extent and kinetics of absorption is not a critical issue.

The oral hypoglycaemic agent used according to the invention can be any oral agent exhibiting a glucose lowering effect. Among these agents, those acting on the ATP-dependent potassium channel of the ß-cells are preferred such as glibenclamide, glipizide, gliclazide and AG-EE 623 ZW. The peptides according to the invention may also advantageously be applied in combination with other oral agents such as metformin and related compounds or glucosidase inhibitors as, for example, acarbose.

The features disclosed in the present description, examples and claims may, both separately and in any combination thereof, be material for realizing this invention in diverse forms thereof. The invention is further illustrated by the following examples which are not to be construed as limiting, but merely as an illustration of some preferred features of the invention.

EXAMPLE 1

Synergistic effect of GLP-1(7-36)amide and glibenclamide in NIDDM patients.

Assays

Blood samples were collected in plastic tubes containing EDTA (0.048 ml, 0.34 M) and Trasylol® (1000 IU Kallikrein inhibitor, obtained from Bayer, West Germany) and immediately placed on ice. The samples were centrifuged at 4°C and the plasma was stored at -20°C. Blood glucose was measured by a glucose oxidase method according to A.S. Hugget and D.A. Nixon, Lancet 2 (1957) 368-370. Plasma C-peptide concentrations were determined by radioimmunoassay (RIA) using a commercially available kit (Novo Research Institute, Denmark). Plasma glucagon concentrations were measured by RIA using antibody 30K as described by G.R. Faloona and R.H. Unger in B.M. Jaffe and Behrman, eds.

Methods of Hormone Radioimmunoassay, Academic Press, New York (1974) 317-330.

For further experimental details (e.g. on calculation of isoglycaemic meal-related insulin response, IMIR), reference is made to M. Gutniak, C. Ørskov, J.J. Holst, B. Ahren and S. efendic, The New England Journal of Medicine **326** (29) (1992) 1316-1322, where a different experiment performed under similar conditions is described.

Methods

On four different days the effect of either injecting glibenclamide, 1 mg i.v., or infusing GLP-1(7-36)amide at a rate of 0.75 pmol per kilogram of body weight per minute or a combination thereof was studied in the same group of 6 insulin treated obese NIDDM patients (Body Mass Index: 30.1±2.4 kg/m^2) and compared to administration of saline as control. Ordinary administration of insulin was stopped 24 hours before the administration of the test compounds or of the saline started and all subjects were fasted overnight. A Biostator (Miles, Diagnostic Division, Elkhart, Ind.) was used for insulin administration in this period in order to normalize blood glucose levels before the administration of the test compounds was initiated and also to keep a normal postprandial blood glucose pattern 180 minutes following the ingestion of a standard test meal comprising boiled potatoes, boiled beef, cooked carrots, a glass of milk containing 0.5% butterfat, and a slice of bread baked from a mixture of wheat and rye flours. In this meal, 28, 26, and 46% of the energy comes from protein, fat and carbohydrates, respectively. Administration of the test compounds was performed (glibenclamide, saline) or initiated (GLP-1(7-36)amide, respectively, 30 minutes after normoglycaemia was achieved. The infusion of (GLP-1(7-36)amide was continued for 210 minutes. After 30 minutes (time zero), the subjects were given the test meal which was consumed within 15 minutes. Blood samples were obtained at -30, 0, 15, 30, 90, 120, 150 and 180 minutes.

Results

After the ingestion of the meal, meal-related C-peptide response, glucagon response and isoglycaemic meal-related insulin requirement (IMIR) was measured. The results are summerized in Table 1.

Table 1

	C-peptide response (pg/ml/210 min)	Glucagon response (pg/ml/210 min)	IMIR (U)
Control (saline)	7.4±3.6	269345±6299	17.4±2.8
GLP-1(7-36)amide	25±9.8	10451±5126	6.3±2.0
glibenclamide	105±53.9	*)	8.3±1.0
GLP-1(7-36)amide + glibenclamide	184±55.1	2526±4873	2.7±0.7

*) glibenclamide had no significant influence on glucagon release.

As indicated in the table, both GLP-1(7-36)amide and glibenclamide significantly increased meal-related C-peptide response ($p<0.02$) and when administered in combination exerted a clear synergistic effect. GLP-1(7-36)amide suppressed the glucagon response ($p<0.01$) while glibenclamide had no significant effect. However, in combination with GLP-1(7-36)amide the glucagon response was almost abolished. Finally, both glibenclamide and GLP-1(7-36)amide lowered IMIR and in combination IMIR was as low as 2.7±0.7.

In summary, this experiment demonstrates a strong synergistic effect of a combination of GLP-1(7-36)amide and glibenclamide.

EXAMPLE 2

Synergistic effect of GLP-1(7-36)amide and glibenclamide in NIDDM patients with secondary failure to sulfonylurea treatment.

Methods.

Eight patients with NIDDM and secondary failure to sulfonylurea treatment participated in the study (age 57.6 ± 2.7 years, body mass index 28.7 ± 1.5 kg/m^2, diabetes duration 7.6 ± 1.2 years, HbA_{1c} 5.8 ± 0.5). The diabetic patients fulfilled the criteria for NIDDM and IDDM according to the USA National Diabetes Data Group. None of the patients had impaired renal function, automatic neuropathy, or proliferative retinopathy, and all had normal liver function. They were instructed to eat a standard diet for diabetic patients at least 2 weeks before and during the study. The patients treated with sulfonylureas stopped their medication one week before the experiments. Those who were treated with insulin were instructed to stop the injections of NPH insulin 24 hours before the studies. Blood glucose concentrations were controlled with subcutaneous injections of regular insulin.

All the subjects were studied after an overnight fast. At 07.30 h on the morning of each study, three cannulas were inserted. One cannula was placed in an antecubital vein and was used to sample blood intermittently for hormone assays. It was flushed with saline after each sampling. A second cannula inserted retro-gradely in a dorsal hand vein was used for continuous monitoring of blood glucose concentrations. The venous blood was arterialized by heating the forearm and hand in a thermoregulated sleeve (Kanthal Medical Heating AB, Stockholm,, Sweden) at 45°C . The third cannula was inserted in the contralateral antecubital vein and was used for all infusions. From approximately 08.00 hours, the patients were connected to a Biostator in order to normalize their blood glucose

concentrations. The algorithm of the Biostator was adjusted in order to normalize basal blood glucose levels. The target for blood glucose concentrations was 4-5 mmol/L. When the target was reached, the Biostator algorithm was changed to monitoring and the feedback insulin infusion was stopped. The experiments were started 30 minutes after normoglycemia was achieved, approximately 90 minutes after connection to the Biostator. An infusion of saline or 0.75 pmol/kg/min of GLP-1(7-36)amide (Peninsula Laboratories, St. Helens, Merseyside, England) then was started and continued for 210 minutes. In glibenclamide experiments an i.v. injection of 1 mg glibenclamide (Hoechst AG, Germany) was given at the same time point. These four studies were performed in a random order with 2-4 weeks elapsed between the experiments. At time 0 the subjects were given a standard lunch, as described in Example 1 which they ate within 15 minutes while sitting in bed. Blood samples were taken at FV, -60, -30, -15, 0, 15, 30, 90, 120, 150, and 180 minutes. Blood glucose was measured continuously.

Results.

In the basal state, the effect on blood glucose and C-peptide levels was monitored 45 minutes after administration of GLP-1(7-36)amide, glibenclamide or a combination thereof had started. The results are summarized in Table 2.

Table 2

	Blood glucose mmol/l	C-peptide pmol/l
Control (saline)	6.0 ± 0.3	0.53 ± 0.06
GLP-1(7-36)amide	5.1 ± 0.4	0.63 ± 0.1
glibenclamide	6.0 ± 0.3	0.56 ± 0.007
GLP-1(7-36)amide + glibenclamide	4.5 ± 0.1	0.72 ± 0.1

These results clearly demonstrates the synergistic effect of the two compounds as glibenclamide had no significant effect on its own while the effect of the combination of GLP-1(7-36)amide and glibenclamide, clearely, exeeded that of GLP-1(7-36)amide alone.

After the ingestion of the meal, the insulinogenic indices (integrated insulin/integrated glucose response) were calculated, again highlighting the synergistic effect of the two compounds, a shown in Table 3.

Table 3

	Insulinogenic index
Control (saline)	1.6 ± 0.6
GLP-1(7-36)amide	21.0 ± 7.2,
glibenclamide	10.6 ± 2.8,
GLP-1(7-36)amide + glibenclamide	37.5 ± 9

CLAIMS

1. Use of GLP-1(7-37), GLP-1(7-36)amide, or a pharmaceutically acceptable peptide containing a fragment of the GLP-1(7-37) sequence, or an analogue or a functional derivative of such a peptide for the preparation of a medicament for use in the treatment of diabetes in a regimen which additionally comprises treatment with an oral hypoglycaemic agent.

2. Use according to claim 1 of GLP-1(7-37) or GLP-1 (7-36)amide.

3. Use according to claim 1 or 2 when the oral hypoglycaemic agent is a blocker of the ATP-dependent potassium channel on β-cells.

4. Use according to claim 1 or 2 when the oral hypoglycaemic agent is a sulfonylurea.

5. Use according to claim 1 or 2 when the oral hypoglycaemic agent is (S)-(+)-2-ethoxy-4-[2-[[3-methyl-1-[2-(1-piperidinyl)phenyl]butyl]amino]-2-oxoethyl]benzoic acid.

6. Use according to claim 1 or 2 when the oral hypoglycaemic agent is a biguanide.

7. Use according to claim 6 when the oral hypoglycaemic agent is metformin.

8. A method of treating diabetes which method comprises administering an effective amount of GLP-1(7-37), GLP-1(7-36)amide, or a pharmaceutically acceptable peptide containing a fragment of the GLP-1(7-37) sequence, or an analogue or a functional derivative of such a peptide to a patient in need of such a treatment in a regimen which additionally comprises treatment with an oral hypoglycaemic agent.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 93/00099

A. CLASSIFICATION OF SUBJECT MATTER

IPC5: A61K 37/28
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC5: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

BIOSIS, EMBASE, MEDLINE, WPI, CHEMICAL ABSTRACTS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Diabetes, vol 40(1991):Suppl. 1, page 237A, J.C. Parker et al: "Glucagon-like peptide 1(7-37) and glibenclamide stimulate insulin secretion by different glucose-dependent mechanisms". --	1-7
X	WO, A1, 8706941 (THE GENERAL HOSPITAL CORPORATION), 19 November 1987 (19.11.87) --	1-7
X	WO, A1, 9011296 (THE GENERAL HOSPITAL CORPORATION), 4 October 1990 (04.10.90) --	1-7

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
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"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 June 1993	28 -06- 1993
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Elisabeth Carlborg Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 93/00099

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO, A1, 9111457 (BUCKLEY, DOUGLAS I. ET AL), 8 August 1991 (08.08.91) -- --------	1-7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 93/00099

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 8
because they relate to subject matter not required to be searched by this Authority, namely:

See PCT Rule 39.1(iv): Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

28/05/93

International application No.
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A1- 8706941	19/11/87	EP-A- 0305387	08/03/89
WO-A1- 11296	04/10/90	NONE	
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Description

The present invention relates to the use of peptides of the general formula I

$$R^1—R^2 \qquad (I)$$

wherein R^1 represents

His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Ser-Arg-Arg-Ala-Gln-Asp-,
5 10 15 20

and R^2 represents OH, the peptide chain

-Phe-Val-Gln-Trp-Leu or -Met-Asn-Thr
25

or a corresponding peptide chain or amino acid which is identical with the two last-mentioned peptide chains with the proviso that one or more of the amino acid(s) of the said two last-mentioned peptide chains has/have been omitted, or salts thereof. Compounds of formula I show interesting and surprising pharmacological properties.

Glucagon, a polypeptide hormone consisting of 29 amino acids, is known to possess several pharmacological effects. The use of glucagon for the treatment of hypoglycemia is based upon its metabolic effects. Furthermore, glucagon exerts a spasmolytic effect on smooth muscle and an inhibitory effect on gastric acid secretion. It has now, surprisingly, been found that compounds of formula I as to quantity possess a similar spasmolytic effect and a similar inhibitory effect on gastric acid secretion as that of glucagon, although compounds of formula I show no or minor, negligible metabolic effect. Hence, compounds of formula I are considered superior to glucagon when only a spasmolytic effect or an inhibition of gastric acid secretion is desired.

Glucagon$_{1-21}$, glucagon$_{1-28}$ and des(22—26)glucagon have for instance been found to have almost the same potency as glucagon as regards inhibitor effect on the amplitude of the contractions of the electrically stimulated guinea pig ileum *in vitro*. 10^{-5}M glucagon caused 83±4% ($\bar{X}$±sd, N=3) inhibition compared to 78±5% for glucagon$_{1-21}$. The effects of 10^{-6} M was 50±3% and 52±5%, respectively, and of 10^{-7}M: 27±3% for either peptide.

Furthermore, glucagon$_{1-21}$ has almost the same potency as glucagon with respect to reducing effect on intestinal motility in rabbits *in vivo*. 100 to 200 µg glucagon and 77 to 154 µg glucagon$_{1-21}$ administered intraveously as a bolus to anaesthetised rabbits of 2.5 to 3.0 kg body weight caused an inhibition of intestinal motility beginning 1 minute after the administration and lasting for about 10 minutes.

The metabolic effects of glucagon$_{1-21}$, glucagon$_{1-28}$ and des(22—26)glucagon, as exemplified by their lipolytic effect on rat free fat cells *in vitro* and their effect on the activation of the adenlyate cyclase *in vitro*, are negligible compared with the metabolic effects of glucagon. No metabolic effects have been found after administration to rats *in vivo*.

It has been shown that glucagon releases insulin from the isolated perfused rat pancreas but glucagon$_{1-21}$ has no such effect when it is infused to the same concentration as glucagon. Furthermore, glucagon$_{1-21}$—contrary to glucagon—does not cause hyperglycemia or release insulin *in vivo* in rats.

In cats with chronic gastric fistulas glucagon$_{1-21}$ as well as glucagon inhibit pentagastrin stimulated gastric acid secretion. 1 µg/kg pentagastrin subcutaneously administered to gastric fistula cats caused an increase in gastric acid secretion of 856±71 µEq (Eq designates equivalent) acid ($\bar{X}$±S.E.M., N=18). When 2 µg/kg glucagon$_{1-21}$ was administered subcutaneously at the same time as 1 µg/kg pentagastrin the increase in acid output was only 417±104 µEq acid (N=6).

Glucagon$_{1-21}$ and gluacon are almost equipotent as regards relaxing effect on a submaximally contracted rabbit gall bladder preparation *in vitro*, and both compounds cause an increase in gall flow in rats *in vivo*. When a gall bladder strip was contracted with 0.1 µg/ml cholecystochinin octapeptide 10^{-8}M glucagon caused 39% relaxation and 10^{-6}M glucagon$_{1-21}$ caused 41% relaxation. The ED_{50} value was for both peptides 2.7×10^{-8} M. Therefore, compounds of formula I may have a potential utility in the treatment of biliary tract and—because of their general spasmolytic properties—possibly urinary *calculi* patients. As regards this utility, the fact that compounds of formula I have no or minor, negligible metabolic effect must be a considerable advantage.

Hence, a compound of formula I or a salt thereof may be used as a therapeuticum or a diagnosticum. The indication areas for use of the compounds of formula I and salts thereof in therapy will be, for example, biliary tract and urinary tract *calculi*, spasms in the digestive system and gastro-duodenal ulcers. The indication areas for use of the compounds of formula I and salts thereof for diagnostic purposes will be investigational techniques such as radiology (X-ray examination), endoscopy (direct observation of the gastro-intestinal tract) and hysterosalphingographia.

Compounds of formula I are converted into pharmaceutical preparations and administered, preferably to humans, in analogy with known methods.

Compounds of formula I and salts thereof can, as diagnosticum, be used in analogy with the use of glucagon for the same purpose. Compounds of formula I and salts thereof can be administered intravenously, intramuscularly or subcutaneously at dosages in the range of from about 1 to 1000 µg/kg body weight, preferably from about 10 to 100 µg/kg body weight, although a lower or higher dosage may be administered. The required dosage will depend on the severity of the condition of the patient and the duration of treatment. A higher dosage may be used for biliary tract and urinary tract *calculi* patients and gastro-duodenal ulcer patients and, in these cases, multiple dosages of the compounds may be administered, for example, parenterally (for example as a continuous infusion) or by the nasal or rectal route.

Compounds of formula I may possibly be administered orally, e.g., by the use of special additives.

For the purpose of parenteral administration, compounds of formula I are dissolved in distilled water and the pH-value is adjusted to about 6 to 8. In order to facilitate the lyophilization process resulting in a suitable product lactose could be added to the solution. The solution is sterile filtered and filled in vials. Thereafter, the solutions are lyophilized and the vials are sealed under aseptic conditions.

For the purpose of nasal administration a solution in a nasal spraying device or nebulisator is used. The compounds of formula I are dissolved in distilled water, the pH-value is adjusted to about 6 to 8 by adding sodium phosphate and citric acid as buffer. Sodium chloride, sorbitol and glycerol are used to obtain an isotonic solution with a suitable viscosity. The solution is administered by the use of a suitable nebulisator or plast spray. The solution may be preserved by the use of known preservatives and a known surfactant may be added.

For the purpose of nasal administration by the use of dose aerosol spray the peptides are mixed with suitable constituents and a mixture of halogencarbons, i.e. monofluorotrichloromethane, difluorodichloromethane and tetrafluorodichloroaethane, in order to obtain a mixture with a vapour pressure producing a well defined single dose when the mixture is administered by the use of a dose aerosol spray.

The compounds of formula I are preferably used by nasal administration in a dosage range between about 0.1 and 100 µg/kg body weight, preferably between 1 and 10 µg/kg body weight, per single dose. This dose could be administered several times per day.

For the purpose of rectal administration suppositories are produced by admixing compounds of formula I, with an inactive constituent such as cocoa butter or with a base such as Polysorbate 85, propylene glycol monostearate and white bee's wax.

Compounds of formula I and salts thereof can be prepared by methods which are generally known in peptide synthesis. Briefly, compounds of formula I can be built up from a protected glucagon fragment, e.g. protected glucagon$_{1-15}$, and a protected peptide containing the remaining amino acids of the desired compound of formula I. The preparation of protected glucagon$_{1-15}$ is described in Res.Discl. 1979, 247. Peptides containing more than amino acids Nos. 16—21 in glucagon can be built up from a protected glucagon fragment, e.g. protected glucagon$_{16-21}$ and a protected peptide containing the remaining amino acids. The use of suitable protecting groups and activations during the peptide synthesis is known to the skilled art worker. It is desired to use protecting groups which can easily be removed.

Thus, glucagon$_{1-21}$, glucagon$_{1-26}$ and des(22—26)-glucagon can be prepared by coupling the protected glucagon fragment:

Adoc-His(Adoc)-Ser(Bu^t)-Gln-Gly-Thr(Bu^t)-Phe-Thr(Bu^t)-Ser(Bu^t)-
5
Asp(OBu^t)-Tyr(Bu^t)-Ser(Bu^t)-Lys(Boc)-Tyr(Bu^t)-Leu-Asp(OBu^t)-OH (II)
10 15

with the protected glucagon fragments:

H-Ser(Bu^t)-Arg(HCl)-Arg(HCl)-Ala-Gln-Asp(OBu^t)-OBu^t (III)
20

H-Ser(Bu^t)-Arg(HBr)-Arg(HBr)-Ala-Gln-Asp(OBu^t)-Phe-Val-Gln-Trp-Leu-OBu^t (IV)
20 25

or

H-Ser(Bu^t)-Arg(HBr)-Arg(HBr)-Ala-Gln-Asp(OBu^t)-Met-Asn-Thr(Bu^t)-OBu^t (V)
20

respectively, by the mixed anhydride method using isobutyl chloroformate. The fully protected peptides so obtained can be deprotected under acid conditions, e.g. by treatment with trifluoroacetic acid containing 10% 1,2-ethanedithiol. The crude peptides can be purified by ion-exchange chromatography, e.g. QAE-Sephadex A-25, followed by a desalting procedure, e.g. gel-filtration on Sephadex G-25. The purified peptides can be isolated by lyophilization. The intermediate protected glucagon fragments IV and V can be prepared by coupling, using the mixed anhydride procedure, the protected glucagon fragment:

Bpoc-Ser(Bu^t)-Arg(HBr)-Arg(HBr)-Ala-Gln-Asp(OBu^t)-OH (VI)
20

with the protected glucagon fragments:

H-Phe-Val-Gln-Trp-Leu-OBu^t (VII)
25

H-Met-Asn-Thr(Bu^t)-OBu^t (VIII)

respectively, whereupon the N-terminal Bpoc group can be removed selectively under mild acid conditions, e.g. by treatment with HCl (0.2N) in methanol/N,N-dimethylformamide.

The protected peptide fragments III, VI, VII and VIII were synthesized by stepwise chain elongation applying conventional procedures such as the active ester or mixed anhydrid methods for coupling.

Peptides of formula I, wherein R^2 represents the peptide chain

Phe-Val-Gln-Trp-Leu
25

or

-Met-Asn-Thr

in which one or more amino acid(s) has/have been omitted, can be prepared in a similar manner as described above with the exception that one or more of the amino acid(s) in question has/have been omitted in the protected peptide fragments VII and VIII.

A process for preparing glucagon$_{1-21}$ has been described in J.Biol.Chem. *247*, 2133, by digesting porcine, bovine or sheep glucagon with carboxypeptidase A. Glucagon$_{1-26}$ is known from Metabolism *25*, Suppl. 1, 1315.

A preferred subclass of compounds of formula I is compounds wherein the amino acid sequence is identical with a continuous part of the amino acid sequence of glucagon. As examples of specific compounds, within this class of compounds, compounds of formula I, wherein R^2 is Phe, Val, Gln, Trp, Leu, Met, Asn or Thr, can be mentioned. A preferred compound of formula I is glucagon$_{1-21}$, because it shows superior pharmacological properties and because it can easily be obtained, e.g. from natural glucagon.

Furthermore, the present invention relates to novel compounds of the general formula I'

R^1—R'^2

wherein R^1 is as defined above, and R'^2 has the same meaning as R^2, provided that R'^2 does not represent

-Phe-Val-Gln-Trp-Leu or OH,
25

or a salt thereof.

Briefly, compounds of formula I' may be prepared by treating a compound of the general formula

R^3—R^4-OBu^t (IX)

wherein R^3 represents

Adoc-His(Adoc)-Ser(Bu^t)-Gln-Gly-Thr(Bu^t)-Phe-Thr(Bu^t)-
5

Ser(Bu^t)-Asp(OBu^t)-Tyr(Bu^t)-Ser(Bu^t)-Lys(Boc)-Tyr(Bu^t)-Leu-
10

Asp(OBu^t)-Ser(Bu^t)-Arg(HX)-Arg(HX)-Ala-Gln-Asp(OBu^t)-,
15 20

R^4 represents the peptide moiety

-Phe-Val-Gln-Trp-Leu-
25

from which one or more of the amino acid(s) has/have been omitted, the peptide moiety

-Met-Asn-Thr(Bu^t)-

or corresponding peptide moieties which are identical with said moiety with the proviso that one or more of the amino acid(s) has/have been omitted, and X represents chlorine or bromine, with an acid such as trifluoroacetic acid.

As examples of salts of compounds of formula I, for example sodium, potassium, magnesium, calcium and zink salts and acid addition salts with organic or inorganic acids such as formic acid, methanesulfonic acid, hydrochloric acid and sulphuric acid can be mentioned. Preferred salts of compounds of formula I are physiologically and pharmaceutically acceptable salts.

The present invention also relates to a pharmaceutical composition comprising a compound of formula I or a salt thereof and one or more pharmaceutically acceptable carrier(s), diluent(s) preferably water, and/or excipient(s). As examples of such carriers conventional preservatives, e.g. methyl or propyl p-hydroxybenzoate, and sodium chloride can be mentioned.

Any novel feature or combination of features described herein is considered essential.

The nomenclature used herein complies with that stated in J. Biol.Chem. *247*, 977, and Biochem. J. *104*, 17. However, for the sake of brevity, glucagon-(1—21)-heneicosapeptide herein has been designated glucagon$_{1-21}$, glucagon-(1—26)-hexacosapeptide has been designated glucagon$_{1-26}$ and des-pentapeptide-(22—26)-glucagon has been designated des(22—26)glucagon. Bpoc represents 1-(biphenyl-4-yl)-1-methyl-ethoxycarbonyl, Adoc represents 1-adamantyloxycarbonyl, Bu^t represents tertiary butyl, and Boc represents tert-butyloxycarbonyl.

The following examples which, however, are not considered to be limiting are presented to illustrate the invention.

Example 1

des(22—26)glucagon

1 g of

Adoc-His(Adoc)-Ser(Bu^t)-Gln-Gly-Thr(Bu^t)-Phe-Thr(Bu^t)-
5

-Ser(Bu^t)-Asp(OBu^t)-Tyr(Bu^t)-Ser(Bu^t)-Lys(Boc)-Tyr(Bu^t)-Leu-
10

-Asp(OBu^t)-Ser(Bu^t)-Arg(HBr)-Arg(HBr)-Ala-Gln-Asp(OBu^t)-Met-Asn-Thr(Bu^t)-OBu^t
15 20

is dissolved in 25 ml of trifluoroacetic acid containing 10% 1,2-ethanedithiol and the reaction mixture is left at 15°C for 3 hours. Thereafter, 200 ml of tetrahydrofuran is added slowly and the precipitate is isolated, washed with tetrahydrofuran and dried *in vacuo*. The resulting product may be purified by ion-exchange chromatography on QAE Sephadex A-25 and desalted by gel-filtration on Sephadex G-25.

Example 2

A preparation for parenteral administration containing 1 mg of glucagon$_{1-21}$ per ml may be prepared as follows:

1 g of glucagon$_{1-21}$ and 99 g of lactose are dissolved in 1 litre of distilled water and the pH-value is adjusted to 7.0. The solution is thereafter sterile filtered. The sterile solution is filled in 10 ml vials in such a way that each vial contains 1.0 ml of the solution. Thereafter, the solutions are lyophilized and the vials are sealed under aseptic conditions.

The preparation in any of the vials is to be dissolved in 1.0 ml of sterile, isotonic water before administration.

Example 3

A preparation for parenteral administration containing 10 mg of glucagon$_{1-21}$ per ml may be prepared as follows:

10 g of glucagon$_{1-21}$ and 90 g of lactose are dissolved in 1 litre of distilled water and the solution is prepared analogously to the method described in Example 2.

Example 4

Rectal suppositories are prepared by admixing 1 mg of glucagon$_{1-21}$ with 4 g of cocoa butter.

Example 5

A nasal plast spray may be prepared as follows:

0.5 g of glucagon$_{1-21}$ is dissolved in about 95 ml of 0.01 M phosphate buffer (pH-value: 7.4) which is made isotonic by the addition of glycerol. The solution is preserved by the addition of 0.01% benzalkonium chloride and 0.05% EDTA whereafter 0.5% polyoxysorbate is added. An isotonic phosphate buffer is added in order to give a resulting volume of 100 ml and the solution is sterile filtered. 15 ml of said solution is filled in a plast spray giving 0.5 mg of glucagon$_{1-21}$, when activated.

Experiment A: Spasmolytic effect

One male rabbit weighing 2.56 kg was anaesthetized with nembutal after an overnight fast. The position of the balloon used for measurement of intestinal motility was 1 meter from pyrolus in the jejunum. The motility was registered before and after intraveneous administration of 77 µg glucagon$_{1-21}$ in 1 ml 0.9% saline containing 0.1% human serum albumin. The effect obtained was nearly complete atonia of the intestine. The onset of effect was 1 minute after the administration and the duration of effect was 11 minutes.

Experiment B: Spasmolytic effect

A male rabbit weighing 2.32 kg was treated as described in Experiment A with the following dosages:

77 µg glucagon$_{1-21}$ in 1 ml of the solution stated in Experiment A intraveneously caused no detectable spasmolytic effect.

154 µg glucagon$_{1-21}$ in 1 ml of the solution in Experiment A intraveneously had a questionable effect.

308 µg glucagon$_{1-21}$ in 1 ml of the above solution had a distinct spasmolytic effect causing nearly complete atonia. The onset of the effect was 2½ minutes and the duration of the effect was 6 minutes.

For comparison glucagon was administered to the same rabbit. 200 µg glucagon intravenously had no detectable effect, however, 400 µg gave a distinct effect comparable to the effect caused by 308 µg glucagon$_{1-21}$.

Experiment C: Gastric acid inhibitory effect

In a male cat weighing approx. 4.5 kg equipped with a cronic gastric fistula the gastric acid secretion was stimulated with 4.5 µg pentagastrin (Peptavlon®) in a volume of 1 ml 0.9% saline containing 0.1% human serum albium subcutaneously in the neck. In 8 experiments 1 ml placebo (0.9% saline with 0.1% human serum albumin) was administered subcutaneously through another cannular in the neck at the same time as the administration of pentagastrin. In 2 experiments 9 µg of glucagon$_{1-21}$ in 1 ml of the above solution was administered simultaneously with the administration of pentagastrin. Gastric acid secretion was collected over periods of 15 minutes and titrated with 0.01N NaOH. The increase in acid secretion after the administration of petagastrin was calculated as µEq acid excreted over 1½ hrs. after the administration subtracting the basal acid secretion before administration of pentagastrin. After administration of 4.5 µg pentagastrin plus placebo the increase in gastric acid secretion was 729±89 µEq acid ($\bar{X}$±S.E.M., N=8). 4.5 µg pentagastrin+9 µg glucagon$_{1-21}$ caused an increase in acid secretion of 238 µEq in one experiment and 231 µEq in another experiment.

Experiment D: Effect on bile flow

In rabbits with catheters in the bile duct the administration of glucagon and glucagon$_{1-21}$ caused a decrease in gall flow immediately after the administration, probably reflecting a decrease in the tonus of the gall bladder. This decrease in flow was followed by an increase in bile flow to quantities higher than before the administration reflecting an increase in production of bile.

One rex rabbit weighing 2.0 kg was equipped with a catheter in the bile duct during nembutal anaesthesia on the day before the experiment. On the day of the experiment the bile was collected for periods of 15 minutes.

The results obtained appear from the following table:

Sampling periods, minutes	0—15	15—30	30—45	45—60	60—75	75—90
Amount of bile, ml	1.20	1.50	1.40	0.20	0.25	3.30

Sampling periods, minutes	90—105	105—120	120—135	135—150	150—165	165—180
Amount of bile, ml	2.80	2.00	0.40	1.65	2.05	3.50

Sampling periods, minutes	180—195	195—210	210—225	225—240	240—255	255—270
Amount of bile, ml	1.10	1.50	1.50	1.35	1.70	1.75

After 45 minutes 200 µg glucagon was administered subcutaneously in 1 ml of 0.9% saline containing 0.1% human serum albumin. After 120 minutes 154 µg glucagon$_{1-21}$ was administered subcutaneously in 1 ml of the above solution. After 195 minutes the placebo (*vide* Experiment C) was administered.

Experiment E: Acute toxicity study

10 mg glucagon$_{1-21}$ administered intraveneously as a bolus to NMRI mice weighing 20 g (i.e. a dose of 500 mg/kg body weight) had no adverse effects. No deaths occurred.

Claims for the Contracting States: BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. A compound for use as a medicament or diagnosticum, of the general formula I

$$R^1—R^2 \qquad (I)$$

wherein R^1 represents

His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Ser-Arg-Arg-Ala-Gln-Asp-
5 10 15 20

and R^2 represents OH, the peptide chain

-Phe-Val-Gln-Trp-Leu
25

or

-Met-Asn-Thr

or a corresponding peptide chain or amino acid which is identical with the two last-mentioned peptide chains with the proviso that one or more of the amino acid(s) of the said two last-mentioned peptide chains has/have been omitted, or a salt of such a compound.

2. A compound according to Claim 1, for use as a medicament or diagnosticum, wherein the compound is used as a spasmolyticum or a gastric acid secretion depressing agent.

3. A compound according to Claim 1 for use as a medicament or diagnosticum, wherein the compound is used for treatment of spasms in the digestive system, for treatment of biliary tract and urinary tract *calculi* and/or for treatment of gastro-duodenal ulcers.

4. A compound according to any one of Claims 1 to 3 for use as a medicament or diagnosticum, wherein R^2 represents OH,

-Phe-Val-Gln-Trp-Leu
25

or

-Met-Asn-Thr.

5. A compound according to any one of Claims 1 to 4 for use as a medicament or diagnosticum, wherein R^2 represents OH.

6. A pharmaceutical composition, which comprises an effective amount of a compound of formula I of Claim 1, or a salt thereof, in association with a suitable physiologically acceptable carrier, diluent and/or excipient.

7. A pharmaceutical composition according to Claim 6, which comprises from 7.5 to 75,000 µg, preferably from 75 to 7500 µg, of a compound of formula I, or salt thereof, per dosage unit.

8. A pharmaceutical composition according to Claim 6 or 7, wherein R^2 represents OH,

-Phe-Val-Gln-Trp-Leu
25

or

-Met-Asn-Thr.

9. A pharmaceutical composition according to Claim 6 or 7, wherein R^2 is OH.

10. A novel compound of the general formula I'

$$R^1—R'^2 \qquad (I')$$

wherein R^1 is as defined in Claim 1 and R'^2 has the same meaning as R^2 as defined in Claim 1, provided that R'^2 does not represent

-Phe-Val, -Phe-Val-Gln-Trp, -Phe-Val-Gln-Trp-Leu
25

or OH, or a salt of such a compound.

Claims for the Contracting State: AT

1. A method of preparing a pharmaceutical preparation for use as a medicament or diagnosticum, which method comprises incorporating in a pharmaceutical preparation a compound of the general formula I

$$R^1—R^2 \qquad (I)$$

wherein R^1 represents

His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Ser-Arg-Arg-Ala-Gln-Asp-
5 10 15 20

and R^2 represents OH, the peptide chain,

-Phe-Val-Gln-Trp-Leu
25

or

-Met-Asn-Thr

or a corresponding peptide chain or amino acid which is identical with the two last-mentioned peptide chains, with the proviso that one or more of the amino acid(s) of the said last-mentioned peptide chains has/have been omitted, or a salt of such a compound.

2. A method according to Claim 1, wherein the pharmaceutical preparation is for use as a spasmolyticum or a gastric acid secretion depressing agent.

3. A method according to Claim 1, wherein the pharmaceutical preparation is for use for treatment of spasms in the digestive system, for treatment of biliary tract and urinary tract *calculi* and/or for treatment of gastro-duodenal ulcers.

4. A method according to any one of Claims 1 to 3, wherein R^2 represents OH,

-Phe-Val-Gln-Trp-Leu
25

or

-Met-Asn-Thr.

5. A method according to any one of Claims 1 to 4, wherein R^2 represents OH.

6. A pharmaceutical composition, which comprises an effective amount of a compound of formula I of Claim 1, or a salt thereof, in association with a suitable physiologically acceptable carrier, diluent and/or excipient.

7. A pharmaceutical composition according to Claim 6, which comprises from 7.5 to 75,000 µg, preferably from 75 to 7500 µg, of a compound of formula I, or salt thereof, per dosage unit.

8. A pharmaceutical composition according to Claim 6 or 7, wherein R^2 represents OH,

-Phe-Val-Gln-Trp-Leu
25

or

-Met-Asn-Thr.

9. A pharmaceutical composition according to Claim 6 or 7, wherein R^2 is OH.

10. A novel compound of the general formula I'

$$R^1—R'^2 \qquad (I')$$

wherein R^1 is as defined in Claim 1 and R'^2 has the same meaning as R^2 as defined in Claim 1, provided that R'^2 does not represent

Phe-Val, Phe-Val-Gln-Trp, -Phe-Val-Gln-Trp-Leu
25

or OH, or a salt of such a compound.

11. A process for the preparation of a compound of the formula I' or a salt thereof, which comprises preparing the same from the parent L-amino acids, whereafter a compound of formula I', if desired, is converted into a salt thereof.

12. A process for the preparation of a compound of the formula I', or a salt thereof, wherein a compound of the general formula

$$R^3—R^4\text{-}OBu^t \qquad (IX)$$

wherein R^3 represents

Adoc-His(Adoc)-Ser(Bu^t)-Gln-Gly-Thr-(Bu^t)-Phe-Thr(Bu^t)-
5

Ser(Bu^t)-Asp(OBu^t)-Tyr(Bu^t)-Ser(Bu^t)-Lys(Boc)-Tyr(Bu^t)-
10

Leu-Asp(OBu^t)-Ser(Bu^t)-Arg(HX)-Arg(HX)-Ala-Gln-Asp(OBu^t)-,
15 20

R^4 represents the peptide moiety

-Phe-Val-Gln-Trp-Leu-
25

from which one or more of the amino acid(s) has/have been omitted, the peptide moiety -Met-Asn-Thr(Bu^t)- or corresponding peptide moieties which are identical with said moiety with the proviso that one or more of the amino acid(s) has/have been omitted, and X represents chlorine or bromine, is treated with an acid, such as trifluoroacetic acid, whereafter the resulting compound, if desired, is converted into a salt thereof.

Patentansprüche für die Vertragsstaaten: BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Verbindung der allgemeinen Formel I,

$$R^1—R^2 \qquad (I)$$

zur Verwendung als Arzneimittel oder als Diagnostikum, worin R^1

His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Ser-Arg-Arg-Ala-Gln-Asp-
5 10 15 20

bedeutet und R^2 OH, die Peptidkette

-Phe-Val-Gln-Trp-Leu
25

oder

-Met-Asn-Thr

oder eine entsprechende Peptidkette oder Aminosäure, die mit den beiden letztgenannten Peptidketten identisch ist, mit der Massgabe, dass eine oder mehrere Aminosäuren der zwei letztgenannten Peptidketten weggelassen sind, bedeutet, oder ein Salz einer solchen Verbindung.

2. Verbindung nach Anspruch 1 zur Verwendung als Arzneimittel oder als Diagnostikum, wobei die Verbindung als Spasmolytikum oder als Mittel zur Hemmung der Magensäuresekretion verwendet wird.

3. Verbindung nach Anspruch 1 zur Verwendung als Arzneimittel oder als Diagnostikum, wobei die Verbindung zur Behandlung von Spasmen im Verdauungstrakt, zur Behandlung von Steinbildungen im Gallen- und Harntrakt und/oder zur Behandlung von Magen- und/oder Zwölffingerdarm-Geschwüren verwendet wird.

4. Verbindung nach einem der Ansprüche 1 bis 3 zur Verwendung als Arzneimittel oder als Diagnostikum, worin R^2 OH,

-Phe-Val-Gln-Trp-Leu
25

oder

-Met-Asn-Thr

bedeutet.

5. Verbindung nach einem der Ansprüche 1 bis 4 zur Verwendung als Arzneimittel oder als Diagnostikum, worin R^2 OH bedeutet.

6. Pharmazeutische Zubereitung, welche ein wirksame Menge einer Verbindung der Formel (I) nach Anspruch 1 oder ein Salz davon in Verbindung mit einem geeigneten physiologisch annehmbaren Träger, Verdünnungsmittel und/oder Excipiens enthält.

7. Pharmazeutische Zubereitung nach Anspruch 6, welche zwischen 7,5 und 75'000 µg, vorzugsweise zwischen 75 und 7500 µg, einer Verbindung der Formel (I) oder eines Salzes davon je Dosierungseinheit enthält.

8. Pharmazeutische Zubereitung nach Anspruch 6 oder 7, worin R^2 OH,

-Phe-Val-Gln-Trp-Leu
25

oder

-Met-Asn-Thr

bedeutet.

9. Pharmazeutische Zubereitung nach Anspruch 6 oder 7, worin R^2 OH bedeutet.

10. Neue Verbindung der Formel I'

$$R^1—R'^2 \qquad (I')$$

worin

R^1 die gleiche Bedeutung hat wie im Anspruch 1 und

R'^2 die gleiche Bedeutung wie die Bedeutung von R^2 im Anspruch 1 hat, mit der Massgabe, dass R'^2 nicht -Phe-Val, -Phe-Val-Gln-Trp,

-Phe-Val-Gln-Trp-Leu
25

oder OH oder ein Salz einer solchen Verbindung bedeutet.

Patentansprüche für den Vertragsstaat: AT

1. Verfahren zur Herstellung einer pharmazeutischen Zubereitung zur Verwendung als Arzneimittel oder Diagnostikum, welches Verfahren die Zugabe einer Verbindung der allgemeinen Formel I

$$R^1—R^2 \qquad (I)$$

worin R^1

His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Ser-Arg-Arg-Ala-Gln-Asp-
5 10 15 20

bedeutet und

R^2 OH, die Peptidkette

-Phe-Val-Gln-Trp-Leu
25

oder -Met-Asn-Thr oder eine entsprechende Peptidkette oder Aminosäure, die mit den beiden letztgenannten Peptidketten identisch ist, mit der Massgabe, dass eine oder mehrere Aminosäuren der zwei letztgenannten Peptidketten weggelassen sind, bedeutet, oder eines Salzes einer solchen Verbindung zu einer pharmazeutischen Zubereitung umfasst.

2. Verfahren nach Anspruch 1, wobei die pharmazeutische Zubereitung als Spasmolytikum oder als Mittel zur Hemmung der Magensäuresekretion verwendet wird.

3. Verfahren nach Anspruch 1, wobei die pharmazeutische Zubereitung zur Verwendung zur Behandlung von Spasmen im Verdauungstrakt, zur Behandlung von Steinbildungen im Gallen- und Harntrakt und/oder zur Behandlung von Magen- und/oder Zwölffingerdarm-Geschwüren vorgesehen ist.

4. Verfahren nach einem der Ansprüche 1 bis 3, worin R^2 OH,

-Phe-Val-Gln-Trp-Leu
25

oder

-Met-Asn-Thr

bedeutet.

5. Verfahren nach einem der Ansprüche 1 bis 4, worin R^2 OH bedeutet.

6. Pharmazeutische Zubereitung, welche eine wirksame Menge einer Verbindung der Formel (I) nach Anspruch 1 oder ein Salz davon in Verbindung mit einem geeigneten physiologisch annehmbaren Träger, Verdünnungsmittel und/oder Excipiens enthält.

7. Pharmazeutische Zubereitung nach Anspruch 6, welche zwischen 7,5 und 75'000 µg, vorzugsweise zwischen 75 und 7500 µg, einer Verbindung der Formel (I) oder eines Salzes davon je Dosierungseinheit enthält.

8. Pharmazeutisch Zubereitung nach Anspruch 6 oder 7, worin R^2 OH,

-Phe-Val-Gln-Trp-Leu
25

oder

-Met-Asn-Thr

bedeutet.

9. Pharmazeutische Zubereitung nach Anspruch 6 oder 7, worin R^2 OH bedeutet.

10. Neue Verbindung der Formel I'

$$R^1—R'^2 \qquad (I')$$

worin

R^1 die gleiche Bedeutung hat wie im Anspruch 1 und

R'^2 die gleiche Bedeutung wie die Bedeutung von R^2 im Anspruch 1 hat, mit der Massgabe, dass R'^2 nicht -Phe-Val, -Phe-Val-Gln-Trp,

-Phe-Val-Gln-Trp-Leu
25

oder OH oder ein Salz einer solchen Verbindung bedeutet.

11. Verfahren zur Herstellung einer Verbindung der Formel (I') oder eines Salzes davon, umfassend die Herstellung dieser Verbindung, ausgehend von den Stamm-L-Aminosäure, worauf eine Verbindung der Formel (I'), sofern erwünscht, in ein Salz davon umgewandelt wird.

12. Verfahren zur Herstellung einer Verbindung der Formel (I') oder eines Salzes davon, bei welchem eine Verbindung der allgemeinen Formel (IX)

$$R^3—R^4\text{-}OBu^t \qquad (IX)$$

worin
R^3

Adoc-His(Adoc)-Ser(Bu^t)-Gln-Gly-Thr(Bu^t)-Phe-Thr(Bu^t)-Ser(Bu^t)-Asp(OBu^t)-Tyr(Bu^t)-
5 10

Ser(Bu^t)-Lys(Boc)-Tyr(Bu^t)-Leu-Asp(OBu^t)- Ser(Bu^t)-Arg(HX)-Arg(HX)-Ala-Gln-Asp(OBu^t)-,
15 20

R^4 den Peptidanteil

-Phe-Val-Gln-Trp-Leu-,
25

von dem eine oder mehrere Aminosäuren weggelassen sind, den Peptidanteil -Met-Asn-Thr(Bu^t)- oder entsprechende Peptidanteile, welche mit den genannten Anteilen identisch sind mit der Massgabe, dass eine oder mehrere Aminosäuren weggelassen sind, und X chlor oder Brom bedeuten,
mit einer Säure wie Trifluoressigsäure behandelt und danach gewünschtenfalls die resultierende Verbindung in ein Salz davon umgewandelt wird.

Revendications pour les Etats Contractants: BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Composé de formule générale I

$$R^1—R^2 \qquad (I)$$

à titre de médicament ou d'agent diagnostique, dans laquelle R^1 représente

His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr- Ser-Lys-Tyr-Leu-Asp-Ser-Arg-Arg-Ala-Gln-Asp-,
5 10 15 20

et R^2 représente OH, la chaîne peptidique

-Phe-Val-Gln-Trp-Leu
25

ou -Met-Asn-Thr ou une chaîne peptidique correspondante ou un amino-acide qui est identique aux deux chaînes peptidiques mentionnées en dernier lieu mis à part le fait que l'un ou plusieurs des acides aminés des deux chaînes peptidiques mentionnées en dernier lieu a/ont été omis, ou un sel de ceux-ci.

2. Composé selon la revendication 1, utilisé à titre de médicament ou d'agent diagnostique caractérisé en ce que le composé est utilisé en tant qu'agent spasmolytique ou en tant qu'inhibiteur de la sécrétion d'acide gastrique.

3. Composé selon la revendication 1, utilisé à titre de médicament ou d'agent diagnostique caractérisé en ce que le composé est utilisé pour le traitement de spasmes du système digestif, pour le traitement du tractus biliaire et des calculs des voies urinaires et/ou pour le traitement des ulcères dans l'appareil gastro-duodénal.

4. Composé selon l'une quelconque des revendications 1 à 3, utilisé à titre de médicament ou d'agent diagnostique, caractérisé en ce que R^2 représente OH,

-Phe-Val-Gln-Trp-Leu
25

ou

-Met-Asn-Thr.

5. Composé selon l'une quelconque des revendications 1 à 3, utilisé à titre de médicament ou d'agent diagnostique, caractérisé en ce que R^2 représente OH.

6. Composition pharmaceutiique contenant une quantité efficace du composé de formule I de la revendication 1 ou un sel de celui-ci, en association avec un support adéquat, physiologiquement acceptable, un diluant et/ou un excipient.

7. Composition pharmaceutique selon la revendication 6 contenant 7,5 à 75 000 µg, de préférence 75 à 7500 µg du composé de formule I, ou un sel de celui-ci, par dose unitaire.

8. Composition pharmaceutique selon la revendication 6 ou 7 dans laquelle R^2 représente OH,

-Phe-Val-Gln-Trp-Leu
25

ou

-Met-Asn-Thr.

9. Composition pharmaceutique selon la revendication 6 ou 7 dans laquelle R^2 est OH.

10. Composé nouveau de formule générale I'

$$R^1—R'^2 \qquad (I')$$

dans laquelle R^1 a la même signification que celle définie à la revendication 1, et R'^2 a la même signification que R^2 à la revendication 1, mis à part le fait que R'^2 ne représente pas Phe-Val, Phe-Val-Gln-Trp,

$$\text{Phe-Val-Gln-Trp-Leu}_{25}$$

ou OH, ou un sel d'un tel composé.

Revendications pour l'état Contractant: AT

1. Procédé de fabrication d'une préparation pharmaceutique destinée à être utilisée comme médicament ou comme agent diagnostique, ledit procédé consistant à incorporer, dans une préparation pharmaceutique, un composé de formule générale I

$$R^1—R^2 \qquad (I)$$

dans laquelle R^1 représente

$$\text{His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-}_{10}\text{Tyr-Ser-Lys-Tyr-Leu-Asp-Ser-Arg-}_{15}\text{Arg-Ala-Gln-Asp-}_{20},$$

et R^2 représente OH, la chaine peptidique

$$\text{-Phe-Val-Gln-Trp-Leu}_{25}$$

ou -Met-Asn-Thr ou une chaîne peptidique correspondante ou un aminoacide qui est identique aux deux chaînes peptidiques mentionnées en dernier lieu mis à part le fait que l'un ou plusieurs des acides aminés des deux chaînes peptidiques mentionnées en dernier lieu a/ont été omis, ou un sel de ces composés.

2. Procédé selon la revendication 1, dans lequel la préparation pharmaceutique est utilisée en tant qu'agent spasmolytique ou en tant qu'inhibiteur de la sécrétion d'acide gastrique.

3. Procédé selon la revendication 1, dans lequel la préparation pharmaceutique est utilisée pour le traitement de spasmes du système digestif, pour le traitement du tractus biliaire et des calculs des voies urinaires et/ou pour le traitement des ulcères dans l'appareil gastro-duodénal.

4. Procédé selon l'une quelconque des revendications 1 à 3 dans lequel R^2 représente OH, -Phe-Val-Gln-Trp-Leu ou -Met-Asn-Thr.

5. Procédé selon l'une quelconque des revendications 1 à 4 dans lequel R^2 représente OH.

6. Composition pharmaceutique contenant une quantité efficace du composé de formule I de la revendication 1 ou un sel de celui-ci, en association avec un support adéquat, physiologiquement acceptable, un diluant et/ou un excipient.

7. Composition pharmaceutique selon la revendication 6 contenant 7,5 à 75 000 µg, de préférence 75 à 7500 µg d'un composé de formule I, ou un sel de celui-ci, par dose unitaire.

8. Composition pharmaceutique selon la revendication 6 ou 7 dans laquelle R^2 représente OH, -Phe-Val-Gln-Trp-Leu ou -Met-Asn-Thr.

9. Composition pharmaceutique selon la revendication 6 ou 7 dans laquelle R^2 est OH.

10. Composé nouveau de formule générale I':

$$R^1—R'^2 \qquad (I')$$

dans laquelle R^1 a les mêmes significations que celle définie à la revendication 1 et R'^2 a la même signification que R^2 à la revendication 1, mis à part le fait que R'^2 ne représente pas

$$\text{Phe-Val, Phe-Val-Gln-Trp, -Phe-Val-Gln-Trp-Leu}_{25}$$

ou OH, ou un sel d'un tel composé.

11. Procédé de préparation du composé de formule I' ou un sel de celui-ci, consistant à préparer ledit composé à partir du L-aminoacide de départ, après quoi le composé de formule I' est éventuellement transformé en un sel de celui-ci.

12. Procédé de préparation d'un composé de formule I' ou d'un sel de celui-ci, dans lequel un composé de formule générale

$$R^3—R^4\text{-}Obu^t \qquad (IX)$$

dans laquelle R^3 représente

Adoc-His(Adoc)-Ser(Bu^t)-Gln-Gly-Thr(Bu^t)-Phe-
5
Thr(Bu^t)-Ser(Bu^t)-Asp(Obu^t)-Tyr(Bu^t)-Ser(Bu^t)-Lys(Boc)-Tyr (Bu^t)-
10
Leu-Asp(Obu^t)-Ser(Bu^t)-Arg(HX)-Arg(HX)-Ala-Gln-Asp(Obu^t),
15 20

R^4 représente la fraction peptidique

-Phe-Val-Gln-Trp-Leu-
25

dont un ou plusieurs acides aminés a/ont été omis, la fraction peptidique -Met-Asn-Thr(Bu^t)- ou les fractions peptidiques correspondantes qui sont identiques à ladite fraction, mis à part le fait que l'un ou plusieurs des acides aminés a/ont été omis, X représentant le chlore ou le brome, est traitéavec un acide tel que l'acide trifluoroacétique, après quoi le composé obtenu est éventuellement transformé en un sel de celui-ci.

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